International Journal of Bio-Technology and Research (IJBTR)
ISSN (P):2249-6858; ISSN (E):2249-796X
Vol. 8, Issue 6, Dec 2018, 1-10

PRODUCTION AND PURIFICATION OF PECTINASE ENZYME FROM *ASPERGILLUS CANDIDUS* AND ITS APPLICATION ON TEA PROCESSING

M. SHOBANA, M. MEENATCHI & M. MEKALA

Department of Microbiology, Sri Ramakrishna college of Arts and Science for women, Coimbatore, Tamil Nadu, India

ABSTRACT

Enzymes are macromolecular biocatalysts which are commercially used in pharmaceuticals, biofuels, food and beverages and consumer products. The enzyme that hydrolyse pectin substances are called as pectinases or pectinolytic enzyme. The organism such as Bacteria, fungi and yeast are used to produce pectinase enzyme. The pectinase enzyme have been used in several conventional industrial process, such as textile plant fiber processing, tea, coffee, oil extraction, treatment of industrial wastewater. Production of pectinase through micro organism which give more effective and low cost production for industries. In this present research work the spoiled fruits (Apple, citron and Grape) and vegetables (Tomatoes) are collected and extracted. The pectin producing organism is isolated and fermented. Enzyme assay was done for the production of pectinases. The produced pectinase are purified for further application on tea leaf processing.

KEYWORDS: Spoiled Fruits and Vegetables, Vincent's Agar, Pectin, Lougal's Iodine Solution & Tea Leaf

Received: Jun 26, 2018; **Accepted:** Jul 17, 2018; **Published:** Nov 12, 2018; **Paper Id.:** IJBTRDEC20181

Original Article

INTRODUCTION

Enzymes are macromolecular biocatalyst and delicate proteins which are commercially used in pharmaceuticals, chemical production, bio fuels, food beverage and consumer products. The enzymes that hydrolyse pectin are broadly classified as pectinases or pectinolytic enzymes. These pectinase enzymes are heteroginous groups and hydrolyses the ester bond between carboxyl and methyl groups of pectin. Pectin is a jelly-like matrix and had a major constituent of cereals, vegetables, fruits and fibers. Pectin is primarily present in the plant tissue. It contains D-galacturonic acid in the form of macromolecules linked with α-1-4- glycosidic bindings which can easily degraded by pectinolytic enzymes. The fruits like pears, apples, orange, guavas, and other citrus fruits and some vegetables such as tomato, carrot and potatoes have high amount of pectin. The source of pectinase enzyme production is spoiled fruits and vegetables, fruit waste dumping soil and fruit peels. And the pectinase is one of the commercial sector in industrial application. Pectinase enzyme are produced by bacteria, fungi, yeast. When comparative to bacteria, the fungi which produce high yield of pectinase enzyme. Production of pectinase through micro organism which give more effective and low cost production for industries. The waste generated from our homes and agricultural farms with adequate nutrient and the high concentration of various sugars, minerals, vitamins and amino acids could serve as alternative and cheeper source for microbial growth production. Pectinase enzyme has wide variety of applications such as coffee and tea processing, fruits juice extraction and reduce viscosity level in animal feed. In this present research work the spoiled fruits and vegetables (Apples,

citrons, Grapes and Tomatoes) are collected and extracted. The pectin producing organism is isolated and fermented. Enzyme assay was done for the production of pectinases. The produced pectinase were purified for further applications. The purified enzyme was used for tea processing.

MATERIALS AND METHODS

Collection and Extraction of Sample

The spoiled fruits (Apple, Citron and Grape) and vegetable (Tomatoes) were collected from local market in and around Coimbatore. The collected fruits and vegetables were homogenized using mortal and pastel and then the extract was collected.

Isolation of Microorganisms

The each sample extract was serially diluted from 10^{-1} to 10^{-6} 0.1ml of each serially diluted sample was inoculated onto Vincent's agar plate and Pectin agar plate using spread plate technique. Then the plates were incubated at 37^{o}c for 24hours for bacteria and for fungi the plates were further incubated for 5days at room temperature. After incubation bacteria and fungi was isolated from Vincent's agar plate and Pectin agar plate (Kumari *et al*; Jayashankar and Graham 1970).

Screening of Isolated Bacteria and Fungi

Screening method was done by spot inoculation method. The bacteria and fungi were isolated from spoiled fruits and vegetables. The different morphological colonies of bacteria and fungi were inoculated on to the Vincent's agar plate. These plates were incubated for 24hours at 37^{o}c for bacteria and for fungi 3-4 days at room temperature. After incubation the plates were flooded with Lougal's iodine to observe the zone of inhibition (Venkata *et al.,* 2013). The organism showing maximum zone of inhibition was taken for the further study.

Identification of Fungal Strains

The microscopic staining was done using lacto phenol cotton blue and it was observed under magnification of (40x) and it was confirmed through MALDI-TOF.

Production of Pectinase Enzyme

Preparation of Natural Media

The pectinase enzyme was produced using both natural and synthetic media. The natural media was prepared by 7g of fruits (apple and orange) and vegetable (potato) are taken and separately added into 100ml of sterile distilled water. A loop full of *Aspergillus candidus* was inoculated on to each media (Rashad R. Al-Hindi *et al,* 2011).

Preparation of Synthetic Media

The synthetic media namely solid state fermentation media pH-4.5 (Maldonaldo *et al.,* 1998) was prepared and sterilized. The *Aspergillus candidus* was inoculated. After inoculation both were kept in rotary shaking incubator for 5-7 days at 150rpm. After incubation the enzyme was centrifuged at 5000rpm for 20minutes and the supernatant was collected and used for further analysis.

Partial Purification of Pectinase Enzyme

Each supernatant was collected (From Natural-potato, orange and apple and Synthetic) mixed with of 80% ammonium sulphate and allowed to precipitate. These precipitates were dissolved in the minimum amount of acetate buffer then dialysis bag was filled with the ammonium sulphate treated supernatant and the bags were dipped into the acetate buffer. Then it was kept into deep freezer at 4^{o}c for 18hours. After that the dialyzed enzyme was used for further analysis (Vibha Bhardwaj and Neelam Garg 2012).

Assay of Pectinase Enzyme

Assay by Dinitrosalicylic Acid Reagent Method

Assay for pectinase enzyme activity was done by Dinitrosalicylic acid reagent (DNS) method (Miller, 1959). In each tube 2ml of sodium citrate buffer (pH-5) was added then 1ml of enzyme extract (both supernatant and partially purified enzyme from apple, potato, orange and synthetic media) and 0.2ml of pectin solution (1%) was incubated for 25minutes at 35^{o}c. After incubation1ml of incubated solution were taken and mixed with 0.5ml of sodium carbonate (1M) solution in each test tube finally 3ml of DNS reagent were added and shake it for 10minutes. Kept the solution in water bath then makeup the solution up to 20ml using distilled water. The enzyme activity was measured by spectro photometrically at 570nm. One unit of enzyme which catalyses the formation of 1µmol of galacturonic acid/min.

Assay by Plating Technique

The another assay of pectinase enzyme was done by the well diffusion method (Lalitha *et al*., 2013). The same Vincent's agar were prepared and sterilized. After sterilization poured into the sterile petridish allow to solidification. Then the wells were created by using sterile cork borer. 0.1ml of each supernatant and purified enzyme was added into the well and then kept into incubation for 48hours at 37^{o}c. After incubation the plates were flooded with lougal's iodine to observe the zone of inhibition.

Application of Pectinase Enzyme

Pretreatment of Pectinase

The supernatant was collected from the fermented synthetic and natural medium and it was treated with 99% acetone and incubated for overnight at 4^{o}c. The sample was centrifuged at 5000rpm for 20minutes, and further pellet was collected and mixed with phosphate buffer solution.

The substrate Green tea leafs were collected from Ooty. 1g of substrate was measured. 1ml of solution was collected from phosphate buffer solution and partially purified enzyme and added to the substrate. Finally incubated at room temperature for 2-3 days. Checked the color and odour of the fermented tea leaf and assay was done by Dinitrosalicylic acid reagent (Jayaraman Angayarkanni and Kishnasamy Swaminathan, 2002).

RESULTS AND DISCUSSIONS

This chapter deals with isolation, screening and identification of fungal strain. The pectinase enzyme partial purification, assay and application were also analyzed.

Isolation of Microorganisms

The isolated bacteria from spoiled citron and tomato showed White, opaque and light yellow color colonies were shown in (Plate 3). The isolated fungi from spoiled apple and grape showed black color spores were shown in (Pate 4).The *Bacillus. Klebsiella and Pseudomonas* were the dominating species in the spoilage of every fruits and vegetables. *Fusarium oxysporum* MTCC 1755 was also obtained from waste apple pomace (Chatanta *et al,* 2008). *Aspergillus niger* is a fungus commonly found on grapes (Chulze, 2006), apples (Oelofse, 2006).

Screening of Isolated Microorganisms

The maximum zone of inhibition was observed when the pectinolytic fungi was treated with Lougal's iodine solution. The zone of inhibition was shown in (Plate 5).The pectinolytic fungi *Aspergillus sp* shows the pectinase activity (K R Amilia *et al,* 2016). The amount of reducing sugar tends to increase when *Aspergillus niger* used as an enzyme producing microorganism (Draginia *et al,* 2007). The high pectinase activity was also showed by *Trichoderma sp* (Ismail A-MS *et al,* 2016).

Identification of Fungi Strain

The species level identification of pectinolytic fungi was confirmed as *Aspergillus candidus* under microscopic observation and MALDI-TOF test. The microscopic observation of *Aspergillus candidus* was shown in (Plate 6) and the MALDI-TOF test results were showed in (Figure 1&2). Based on the morphological characters, pectinolytic fungi were identified as *Aspergillus sp, Trichoderma sp, Penicillum sp and Fusarium sp* (K R Amilia *et al,* 2016).

Production of Pectinase Enzyme

Production of pectinase enzyme using natural and synthetic media. The pectinase enzyme was produced from natural source (apple, orange and potato peels) were showed in (Plate 7,8 & 9). The produced pectinase enzyme from synthetic medium under solid state fermentation was shown in (Plate 10). The higher pectinase production was observed in solid state fermentation (Ramachandran Sandhya and Kurup G, 2013). The polygalacturonases and xylanase had highest level contents in the cell-free broth of tested fungi and important pathogenicity factors for spoilage fungi (Dimatteo *et al,* 2006 and Niturea *et al,* 2008).

Partial Purification of Pectinase Enzyme

The crude enzyme was partially purified by dialysis method. Through the partial purification the pectinase enzymes were separated from other sources. The partially purified pectinase enzyme which gave maximum effect than the crude enzyme

Enzyme Assay

Enzyme Assay by DNS Method

The pectinase activity was measured by DNS. When compare to crude enzyme, the partially purified enzyme (from natural and synthetic source) which shows the high pectinase activity of O.D (570nm) value was shown in (Table 2).

The polygalacturonase, the highest level of activity was detected in the *Aspergillus japonicus* (7433±327 units/100ml) *Aspergillus niger* (4197±209 units/100ml). The orange peel alone shows the better production of polygalacturonase was observed (287.22 units/gds).

Enzyme Assay by Plating Technique

The maximum zone of inhibition indicated high pectinase activity was characterized by Lougal's iodine solution. The zone of inhibition were tabulated and shown in (Table 1). The same results were shown in (Lalitha *et al*, 2013).

Application

The tea leaves which were treated with pectinolytic fungi showed change in color from green to brown. The results were shown in (Plate 11 & 12). The O.D value of pectinase activity was shown in (Table 3).

PLATES

(A) Apple (B) Grape (C) Citron (Tomato)

Plate 1: Collected Spoiled Fruits and Vegetables

(A) Citron (B) Apple (C) Tomato (D) Grape

Plate 2: Extraction of Sample

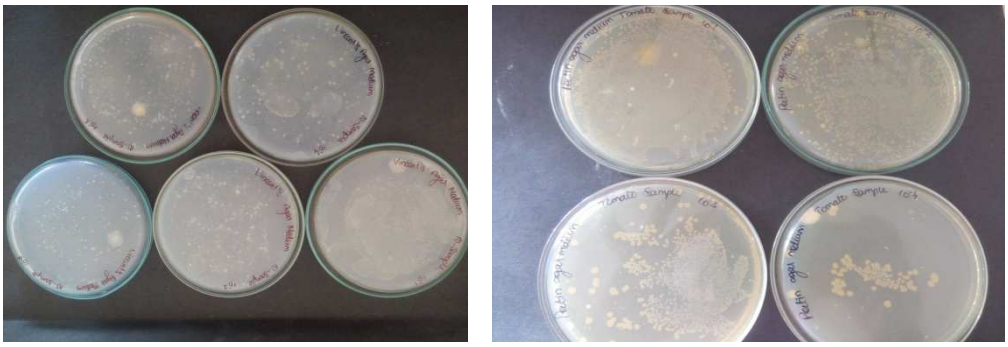

Plate 3: Isolation of Bacteria from Citron and Tomato

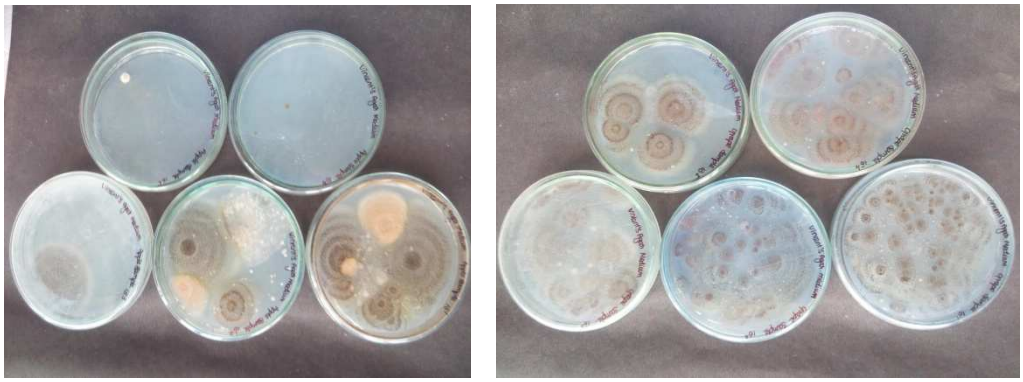

Plate 4: Isolation of Fungi from Apple and Grape

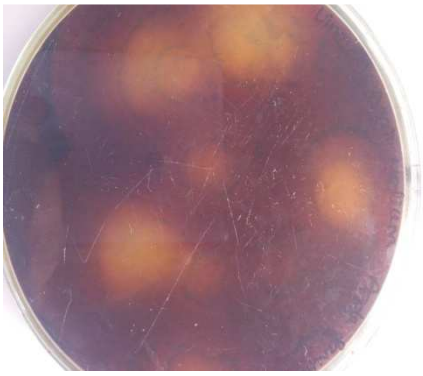

Plate 5: Screening of Isolated Fungi

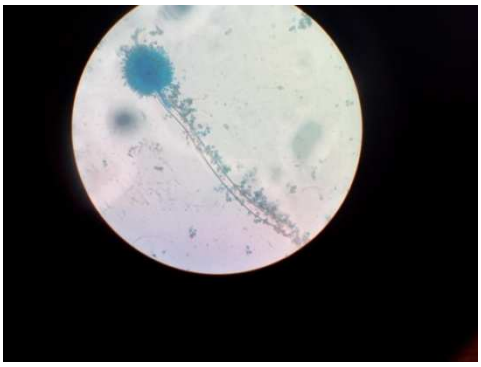

Plate 6: Microscopic Observation of Isolated Fungi

Production of pectinase enzyme from Natural media

(A) Control (B) Production of pectinase enzyme

Plate 7: Source from Apple

Plate 8: Source from Orange

Plate 9: Source from Potato

(A) Control (B) production of Pectinase enzyme.

Plate 10: Production of Pectinase Enzyme from Synthetic Media

(A) Control (B) Crude enzyme of natural (C) Purified enzyme of natural

(D) Crude enzyme of synthetic (E) Purified enzyme of synthetic

Plate 11: Application of Pectinase Enyme

(A) Crude enzyme of natural (B) Purified enzyme of natural

(C) Crude enzyme of synthetic (D) Purified enzyme of synthetic

Plate 12: Pectinase Activity by DNS Method

FIGURE

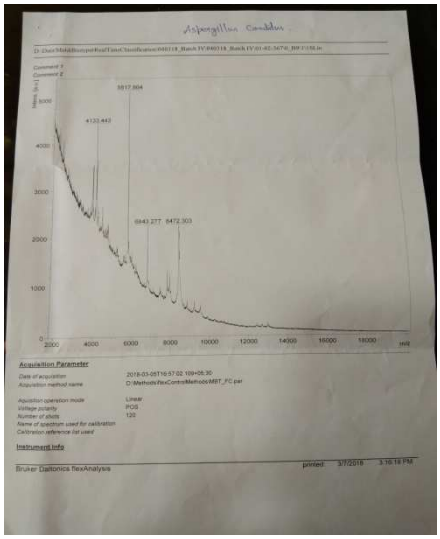

Figure 1: Identification of Fungal strain

Figure 2: By MALDI-TOP test

TABULATION

Table 1: Zone of Inhibition of Pectinase Enzyme

Source	Crude Enzyme	Purified Enzyme
Apple	3mm	5mm
Orange	8mm	13mm
Potato	7mm	11mm
Synthetic	8mm	11mm

Table 2: UV Reading of Pectinase Activity by DNS Method

Source	Crude Enzyme (µ/ml)	Purified Enzyme (µ/ml)
Apple	0.126	0.145
Orange	0.196	0.234
Potato	0.137	0.168
Synthetic	0.114	0.155

Table 3: Application of Pectinase Enzyme in Tea Leaf

Source	Crude Enzyme (µ/ml)	Purified Enzyme (µ/ml)
Natural	0.234	1.013
Synthetic	0.344	0.820

7. CONCLUSIONS

Pectinases are novel enzymes which are integral in various food industry. Utilization of fruit processed industrial by-products and waste as substrate acts to recycle the waste and to decrease the production cost making it economical. There are a lot of industrial processes to which pectinases can be applied to improve the quality and the yield of final products. The bacteria and fungi were isolated from spoiled fruits and vegetables. *Aspergillus candidus* was found to be the potent source for pectinase production. The produced pectinase were purified by dialysis method and the activity of pectinase was done by DNS method. The natural source of pectinase activity which shows more effective than synthetic source. Application of produced pectinase enzyme in Tea leaf was done. The result obtained in the present study will be useful for commercial production of pectinase and other application of fruit juice extraction, bleaching of paper and processing of fibres.

REFERENCES

1. *Anam Tariq and Zakia Latif., (2012). Isolation and Biochemical Characterization of Bacterial Isolates Producing different Levels of Polygalacturonases from Various Sources. African Journal of Microbiology Research. Vol(6): 7259-7264.*
2. *Vibha Bhardwaj, Neelam Garg., (2014). Production, Purification of Pectinase from Bacillus sp. MBRL576 Isolate and its Application in Extraction of Juice. International Journal of Science and Research(IJSR). Vol(3): 648-652.*
3. *Reda, A. Bayoumi, Hesham, M. Yassin, Mahmoud, A.Swelim, Ebtsam, Z. Abdel-All., (2008). Production of Bacterl Pectinase(s) from Agro-Industrial Wastes Under Solid State Fermentation Conditions. Journal of Applied Sciences Research. Vol(4): 1708-1721.*
4. *K. Prathyusha and V. Suneetha., (2011). Bacterial Pectinases and their Potent Biotechnological Application in Fruit Processing/Juice Production Industry. Journal of Phytology. Vol(3): 16-19.*

5. *Abhishek Mathur, Akhilesh Kushwaha, Aman Kumar Singh and Ankur Katiyar., (2014). Isolation, Purification and Characterization of Alkaline Pectinase from Bacillus subtilis Isolated from Soil. Pelagia Research L ibrary. Vol((5): 1-5.*

6. *G.A. Aaisha and D.L., (2016) Barate. Isolation and Identification of Pectinolytic Bacteria from Soil Samples of Akola Region, India. International Journal of Current Microbiology and Applied Sciences. Vol(5): 514-524.*

7. *Ramachandran Andhya and Kurup G., (2013). Screening and Isolation of Pectinase from Fruit and Vegetable wastes and the use of Orange Waste as a Substrate for Pectinase Production. International Research Journal of Biological Sciences. Vol(2): 34-39.*

8. *KR Amila, ALA Sari R Setyaningsih., (2017). Isolation and Screening of Pectinolytic Fungi from Orange (Citrus nobilis Tan.) and Banana (Musa acuminata L) Fruit. International Conference on Food Science and Enigineering. 1-5.*

9. *Rashad R. Al-Hindi, Ahmed R. Al-Najada and Salesh A. Mohamed., (2011). Isolation and Identification of some Fruit Spoilage Fungi: Screening of Plant cell wall degrading enzymes. African Journal of Microbiology Research. Vol 5 (4): 443-448.*

10. *Gyan Datta Tripathi, Zoya Javed, Sushma and Adrash Kumar Singh., (2010). Pectinase Production and Purification from Bacillus subtilis Isolated from Soil. Advances in Applied Science Research. Vol 5 (1): 103-105.*

11. *Ramachandran Sandhya and Kurup G., (2013). Screening and Isolation of Pectinase form Fruit and Vegetable Waste and the Use of Orange Waste as a Substrate for Pectinase Production. International Research Journal of Biological Sciences. Vol. 2(9): 34-39.*

12. *K. Mehraj Pasha, P. Anuradha and D. Subbarao., (2013). Applications of Pectinases in Industrial Sector. International Journal of Pure and Applied Sciences and Technology. Vol 16(1): 89-95.*

13. *Mukesh Sharma, Madhu Rathore, Nevadita Sharma, Sonal Dayama, Babita jogpal, Kirati Dashora and Anju Sharma., (2011). Isolation o f Pectinolytic Bacteria from Rotten Tomato. Journal of Medical and Dental Research. Vol 1(3): 158-161.*

14. *Dr. K. Sridevi, Dr. P. SivaRaagini, M. Sumanth and Dr. K. Vijalakshmi., (2016). Isolation and Screening of Pectinolytic Bacteria from the Mango Fruit Yards. World Journal of Pharmacy and Pharmaceutical Sciences. Vol 5(1): 738-748.*

15. *Prakash S, Karthik R, Tamil venthan M, Sridhar B and Bharath P G., (2014). Optimization and Production of Pectinase from Bacillus subtilis (MTCC 441) by using Orange Peel as a Substrate. International Journal of Recent Scientific Research. Vol 5 (6). 1177-1179.*

16. *Lalitha Chudhary and T.S Dhaka., (2016). Isolation and Identification of Bacteria from some Spoiled Fruits. Plant Archives. Vol 16: 834-838.*

17. *Rokade D.D., Vaidya S.L., Rehman Naziya M.A., Dixit P.P., (2015). Screening of Pectinase Producing Bacteria, Isolated From Osmanabad Fruit Market Soil. International Journal of Interdosciplinary and Multidisciplinary Studies (IJMS). Vol 2: 141-145.*

18. *Nitinkumar P. Patil and Bhushan L. Chaudhari., (2010). Production and Purification by Soil Isolate Penicllim Sp and Search for Better Agro-Residue for Its SSF. Recent Research in Science and Technology. Vol 2 (7): 36-42.*

19. *Arifa Jabeen, Qurat-ul-ain Hanil, Misbah Hussain, Anam Munawar, Nisma Farooq and Shehar Bano., (2015). Screening, Isolation and Identification of Pectinase Producing Bacterial Strains from Rotting Fruits and Determination of Their Pectinolytic Activity. Openaccess Journal. Vol 3(2): 42-45.*

20. *K. Prathyusha and V. Suneetha., (2011). Bacterial Pectinases and Their Potent Biotechnological Application in Fruit Processing/Juice Production Industry: A Review. Journal of Phytology. Vol 3 (6): 16-19.*

21. *Adeleke Bartholomew Saanu. Pectinolytic Activity of Mutagenic Strain of Leuconostoc Mesenteroides Isolated from Orange and Banana Fruit Waste. Journal of Applied Microbiology and Biochemistry. 1-6. Vol 2 (7). 2017.*

22. *Danielle Biscaro Pedrolli, Alexandre Costa Monterio, Eleni Gomes and Eleonora Cano Carmona., (2009). Pectin and Pectinases: Production, Characterization and Industrial Application of Microbial Pectinolytic Enzymes. The Open Biotechnology Journal. Vol 3: 9-18.*

23. *Jansirani.D, Saradha.R, Selvapriyadharshini.J, Salomideborani.N, Halimuthul Nurji.R, Jerin Nivetha.J and Ranjana Devi.B., (2014). Producation of Pectinase by Bacillus sp., Isolated form Soil. National Conference on Green Engineering and Technologies for Sustainable Future. 277-279.*

24. *Ishtiaq Ahmed, Muhammad Anjum Zia, Muhammad Azhar Hussain, Zain Akram, Muhammad Tahir Naveed and Azin Nowrouzi., (2009). Bioprocessing of Citrus Waste Peel for Induced Pectinase Producation by Aspergillus niger; Its Purification and Characterization. Journal of Radiation Research and Applied Sciences. 148-15.*

25. *Kaur, S., & Nigam, V. (2014). Production and application of laccase enzyme in pulp and paper industry. International Journal of Research in Applied, Natural and Social Sciences, 2(4), 153-158.*

26. *Maldonado M.C and Strasser de Saad A.M., (1998). Production of Pectinesterase and Polygalacturonase by Aspergillus niger in Submerged and Solid State Systems. Journal of Indian Microbiological Biotechnology. 34-38.*

27. *Miller G.L., (1959). Use of Dinitrosalicyclic Acid Regent for Determination of Reducing Sugar. Analytical Chemistry.426-428.*

28. *Venkata E., Naga Rajunad, G. Divakar., (2013). Production of Pectinase by Using Bacillus circulans Isolated from Dump Yards of Vegetable Waste. International Journal of Pharmaceutical Science and Research. Vol. 4(7): 2615-2622.*